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ABSTRACT

This document presents a science experiment demonstrating the scientific method. The experiment consists of testing the fermentation capabilities of yeasts under different circumstances. The experiment is supported with computer software called BioLab which demonstrates yeast's response to different environments. (YDS)

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BioLab: Using Yeast Fermentation as a Model for the Scientific Method.

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ASSOCIATION FOR BIOLOGY LABORATORY EDUCATION

BioLab: Using Yeast Fermentation as a Model for the Scientific Method

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Notes to the Instructor
Yeast Fermentation Laboratory

Time Frame:

The activities describe here will be the work of two two-hour laboratory meetings. The exercise could also be modified to use in one lab period.

Method:

This exercise is student designed and computer assisted. In this exercise students learn to use the scientific method by devising their own experiments, then collecting and analyzing their data. In their experiments students test how well yeast cells ferment a variety of substrates (of their choosing) in specific temperature conditions.

Computer Simulation Currently the ***BioLab*** computer program is not available for commercial distribution. A data base for yeast fermentation of different substances is in Appendix E.

The computer simulation, ***BioLab*** , consists of three sections: Validation, Prediction, and Model Experiments. BioLab was designed by the authors. It is hoped that the program will be commercially available in the near future. In the Validation section, the program compares student-collected data with data collected from previous trials run during the writing of this exercise. The Validation experiment "validates" student data by showing them that their data are similar to those in the program. Variations may occur between the students' findings and the simulation's findings and the simulation is still a valid tool. This is the result of slight variations in the experimental conditions each time the work is done. It should also reinforce to the student that the data collected in the laboratory is reliable. In the Prediction experiments, the student can enter projected data, based upon his/her readings and lab work, in an attempt to predict the outcome of a particular experiment. These data are based on students estimates of carbon dioxide output under various experimental conditions emphasizing the different variables: temperature, time, substrate concentration and substrate type. Finally, the Model experiment allows the student to run a variety of experimental simulations comparing different experimental conditions by manipulating a single variable in a relatively short amount of time. This should allow the student to understand how yeast responds under various experimental conditions without actually doing the laboratory experiments. The changing impact of these variables is of particular importance. The extent to which this information may be extrapolated to other organisms and environments is also important to consider.

The instructor should use the computer simulation prior to introducing it to the students. It may take two or three 15-20 minute explorations to feel comfortable with the simulation.

Objectives

Course

1. Creative application of the scientific methods to complex problems as individuals or a part of a team.
2. Proficiency in the critical analysis of and communication about biological topics.

Lesson

1. Integrate theoretical and practical aspects of the laboratory experiment. Specifically, correlate the background reading material and the physical set-up of the experiment.
2. Use the scientific method to devise experimental conditions to test.
3. Compare the experiment performed in the laboratory with the computer simulation.

Procedure/Plan of Presentation

1. Thus far, the students have been introduced to the scientific method and have written an experimental proposal to test a hypothesis. During these two laboratory meetings, students will:

   a. examine yeast fermentation under a variety of conditions
   b. prepare a formal laboratory report to describe their experiments and results
   c. use the computer simulation to validate their work and to explore further options for experimentation
   d. repeat the original experiment or devise an experiment which is more precise or will better tests their hypothesis.
   e. revise the laboratory report to reflect changes in experimental design
2. Demonstrate the computer simulation and explain the parts to the students.
3. "Walk" the students through the laboratory protocol.
4. Give the students the laboratory protocol and directions for accessing the computer simulation during Laboratory meeting 2.
5. Introduce the BioLab software.

Student Outline

Fermentation I

Introduction:

In the laboratory, biologists do not usually have books telling them what experiment to do next. Rather, biologists choose lines of investigation based on their interests and the work of the laboratory. They read papers in journals about the area(s) of biology that interest them and ask questions about what they have read. Often, reading papers will suggest new lines of work or give the scientists new ideas for experiments to do. In this laboratory, biologists will study the fermentation of substrates (food molecules) by yeast cells using respirometers as described in the directions below.

As a new scientist in the lab, your assignment is to formulate a hypothesis to test concerning yeast fermentation. After stating your hypothesis, you will set up a controlled experiment to test your hypothesis. You will then write a formal laboratory report describing your work.

Background:

Yeast are unicellular fungi that are versatile laboratory microorganisms. They grow rapidly and have simple nutritional requirements. When yeast

degrade nutrients in the absence of oxygen they use the process of glycolysis to produce energy in the form of ATP. For millennia, humans have used the alcoholic fermentation capability of yeast of the genus *Saccharomyces* to produce breads, crackers and a variety of fermented beverages including beer and wine. The general equation for the fermentation reaction is:

Substrate + Glycolytic Enzymes ♦ Ethyl Alcohol (C_2H_5OH) + CO_2 + ATP

Notice the substrates and the products in the reaction. What chemicals do you think would be appropriate **substrates** for yeast to degrade? The **glycolytic enzymes** are the enzymes of glycolysis which function under anaerobic conditions.

Potential substrates for this work include sucrose, a disaccharide composed of glucose ($C_6H_{12}O_6$) and fructose, an isomer of glucose. Sodium chloride (NaCl) is table salt and sodium lauryl sulfate ($C_{12}H_{25}O_4SNa$) is an ionic detergent. Detergents are molecules with both positively and negatively charged extremities. Starch or amylose is a polymer of glucose produced by green plants. Sodium saccharin ($C_7H_4NNaO_3S.2H_2O$) is a sugar substitute that is many times sweeter than sucrose. Ethyl alcohol (C_2H_5OH) is a by-product of the fermentation of sugar by yeast.

Now look at the products and try to separate them into useful substances and waste products. What difference might these make in the rate of fermentation? You *need to know more* about the chemicals you test to understand their impact on the fermentative activities of the cell better. Look in chemistry textbooks or a chemical dictionary.

"Carbon dioxide gas accumulates as a waste product of fermentation in yeast and cellular respiration in many kinds of cells, including yours. Fermentation releases two molecules of the gas from the anaerobic (not requiring molecular oxygen) degradation of a substrate, usually glucose, as well as two molecules of ethanol plus usable energy for cell function. Cellular respiration, an aerobic (requiring molecular oxygen) process, liberates six molecules of carbon dioxide as well as several water molecules and energy. More energy is released by cellular respiration than by fermentation because glucose is completely oxidized in the process. Thus, carbon dioxide is a waste product of the energy-releasing mechanisms of the cell. Logically, then, carbon dioxide is an indicator of the rate of substrate degradation in an organism. More carbon dioxide will be released from an organism as the rate of fermentation or cellular respiration increases. What factors in the yeastís environment might change the rate of fermentation or respiration, and thus, the rate of carbon dioxide release? To answer this question, you need to know that both respiration and fermentation are controlled by enzymes within the cells.

As a waste product, carbon dioxide can affect activities of the organism. Increased carbon dioxide levels stimulate more rapid breathing rates in humans which clears carbon dioxide from the system. For an aviator without an oxygen supply, the partial pressure of carbon dioxide (PCO_2) decreases from 40 mm Hg to 24 mm Hg in the alveoli from 0 to 40,000 feet above sea level. During that same change in altitude the partial pressure of oxygen (PO_2) decreases dramatically from 104 mm Hg to 12 mm Hg. An unacclimated individual will breathe more rapidly attempting to take in more oxygen beginning at about 8,000 feet and will reach a maximum breathing rate between 16,000 and 23,000 feet. The individual may also show other symptoms of hypoxia including mental slowness, sleepiness, nausea, giddiness or headache. On the other hand, an aviator breathing pure oxygen can ascend to 47,000 feet before showing the effects of hypoxia.1"

1Guyton, Arthur C. 1986. *Textbook of Medical Physiology* . Seventh Edition. W.B. Saunders Company, Philadelphia. 1057 pp.

Objectives:

1. Creative application of the scientific methods to complex problems as individuals or a part of a team.

2. Proficiency in the critical analysis and communication about biological topics.

Lesson:

1. Frame an experimental question and pose a hypothesis based upon the reading material and your need to validate the ***BioLab*** software as an accurate representation of true experimental conditions for future use.

2. Integrate theoretical and practical aspects of a laboratory experiment. Focus on the relationship between the background reading material and the physical set-up of the experiment to answer your experimental question.

3. Devise experimental conditions to test using the scientific method.

4. Execute an experiment which answers your question and subsequently tests your hypothesis under the experimental conditions you devised.

5. Compare the experimental data from the experiment performed in the laboratory with the computer simulation.

6. Explain the possibilities for variation between your laboratory data and the simulation.

7. Describe the impact of altering variables: time, temperature, substrate concentration and substrate type in the environment of an organism.

8. Predict the impact on an organism of changing the single variables: time, temperature, substrate concentration and substrate type in the environment of an organism.

Materials (for one lab section; 24 students working in groups of 3-4)

- 1 package yeast; Fleischmann's Rapid Rise or Red Star Rapid Rise; 1/4 oz. = 7 g.; check the expiration date.
- 1 one liter flask
- 500 mL distilled water
- 10 mL one-piece plastic pipettes (4-6 per group)
- 16 x 125 mm test tubes (4-6 per group)
- Test tube racks (1 per group)
- Floral clay

- Water bath at 37Y C
- 250 mL flasks or beakers (1 per group, to carry yeast solutions to lab stations)
- Pasteur pipettes (4-6 per group) and pipette bulbs
- Parafilm cut in small squares
- Test solutions in labeled flasks:

a. 10% sucrose
b. 5% sucrose
c. 2.5% sucrose
d. 10% NaCl/sucrose
e. 5% NaCl/sucrose
f. 2.5% NaCl/sucrose
g. 1.25% NaCl/sucrose
h. 2.5% Na lauryl sulfate/sucrose
i. 1.25% Na lauryl sulfate/sucrose
j. 0.67% Na lauryl sulfate/sucrose
k. 0.33% Na lauryl sulfate/sucrose
l. 10% starch
m. 5 % starch
m. 2.5% starch
o. 2.5% Na saccharin
p. 1.25% Na saccharin
q. 0.67% Na saccharin
r. 30% EtOH/sucrose
s. 20% EtOH/sucrose
t. 10% EtOH/sucrose
u. 5% EtOH/sucrose

- Computer and printer with BioLab software loaded and running.

Lab Instructor:

Start the computer and printer. Start ***BioLab*** software. Select the "Validation" section.

Directions:

1. Use small lumps of floral clay to seal the tips of the 10 mL pipettes. Remove the cotton plug from the other end of each pipette.
2. Add approximately 6 mL of yeast solution to the open end of each of the 10 mL pipettes (up to the number 4). Be sure to insert the tip of the Pasteur pipette *below* the constriction to add each solution. This may take some practice.
3. Add enough of the appropriate test solution to fill each pipette (Fisher Brand Disposable Serological pipette) to the double blue lines below the constriction. This should be about 6 mL. Think about what this step does to the concentration of the test solution.
4. Cover the open end of each pipette securely with Parafilm and invert several times to mix the yeast and test solutions.
5. Remove the Parafilm. Invert one of the test tubes over the open end of the pipette and quickly invert both. Your instructor will demonstrate this step. A bubble of air will move into the pipette when you do this.
6. Place your "respirometer" in the test tube rack. Add yeast and test solution to each of the other pipettes and place them in the rack as well.
7. Allow 10 minutes for the respirometers to equilibrate. Take a reading from each pipette at this time (Time 0) and every 5 minutes for 35 to 45 minutes. Remember that each space on the pipette equals 0.1 mL. You want to take the DIFFERENCE between the initial reading (Time 0) and each subsequent reading (mL at Time0 - mL at Time1; mL at Time0 - mL at Time2, etc.).
8. Record your data in the Data Table on this sheet.
9. Input your data into the computer in the "Validation" section of the ***BioLab*** software. Be sure to answer any questions.
10. Print out your product before you close out the software.

Assignment:

Write a formal laboratory report on your experiment as described previously in the laboratory. Supplement your report with using your printout. Be sure to use *references* to describe the substrate(s) used and to help you interpret and explain your results.

Data Table:

Substrate												
Volume	reading	change	reading	change	reading	change	reading	change	reading	change	reading	change
TIME												
0												
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												

Fermentation II

Introduction

In the previous experiment, you focused on the interaction of yeast cells with their environment. The substrate, their concentrations, as well as time and temperature are part of the yeast cells' environment.

In todayís exercise you will critically review your previous laboratory work and then rework one or more parts of the experiment. As a result, you will refine laboratory techniques and correct some of your errors. More importantly, you can revise an experimental design, or begin again with a better experimental plan.

Computer Simulation:

As with the previous laboratory, you will use the Biology Fermentation Experiment.

Lesson Objectives:

Reinforcement from previous Laboratory:

1. Frame an experimental question and pose a hypothesis based upon the reading material and your experience from the previous laboratory.
2. Integrate theoretical and practical aspects of a laboratory experiment. Focus on the relationship between the background reading material and the physical set-up of the experiment to answer your experimental question.
3. Devise experimental conditions to test using the scientific method.
4. Execute an experiment which answers your question and subsequently tests your hypothesis under the experimental conditions you devised.
5. Compare the experimental data from the experiment performed in the laboratory with the computer simulation.
6. Explain the possibilities for variation between your laboratory data and the simulation as well as sources of error.

Enhanced Objectives Using BioLab:

7. Describe how time, temperature, substrate type and concentration affect fermentation. 8. Analyze the results of the computer-based comparison and explain the results in the context of the four variables, the fermentation process and the environmental impact of the variables.

Directions:

1. Restate or modify your hypothesis from the previous laboratory period.
2. Refer to Procedures and Directions in the previous laboratory.
3. Print the results of each experiment, along with your predictions and explanations.

BioLab:

With your computer, use the BioLab software using the Model experiment to compare the impact of the four variables Time, Temperature, Substrate Concentration, and Substrate Type. Specifically focus on these comparisons:

Comparison	Substrates	Temp	Time	Concentration
Substrates:				
1.	Sugar Na lauryl SO_4	45Υ 45Υ	5 min. 5 min.	2.5% 2.5%
2.	Sucrose Starch	37Υ 37Υ	5 min. 5 min.	5.0% 5.0%
3.	Sucrose EtOH	45Υ 45Υ	5 min. 5 min.	10.0% 10.0%
Temperatures:				
4.	Sucrose Sucrose	45Υ 37Υ	5 min. 5 min.	5.0% 5.0%
Concentration:				
5.	EtOH EtOH	45Υ 45Υ	5 min. 5 min.	5.0% 10.0%
6.	NaCl NaCl	45Υ 45Υ	5 min. 5 min.	1.25% 5.0%
Integrated:				
7.	Sucrose EtOH Sucrose EtOH	45Υ 45Υ 37Υ 37Υ	5 min. 5 min. 5 min. 5 min.	10.0% 10.0% 10.0% 10.0%

Assignment:

Write a formal laboratory report on your experiments as described previously in Activity. Supplement your report with your printouts including the results of the eight comparisons done outside the lab. Be sure to use at least 3 *references* to describe the substrate(s) used and to help you interpret and explain your results. Be sure your report explains the results in the context of your selected variable and its impact on the environment of the organism.

Data Table:

Substrate												
Volume	reading	change	reading	change	reading	change	reading	change	reading	change	reading	change
TIME												
0												
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												

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Ambrose, Harrison W, and Katherine Peckham Ambrose. 1987. A handbook of biological investigation. Fourth Edition. Hunter Textbooks, Inc. Knoxville, TN 204 pp. (Pages 189-197 provide some background on laboratory organization, while chapters 1 and 2 explore "what science is" and "asking questions about natural phenomena".)

Browne, M. Neil and Stuart M. Keeley 1994. Asking the right questions: A guide to critical thinking. Prentice-Hall, Englewood Cliffs, NJ 175 pp. (This book is good background reading describing critical thinking in the classroom setting.)

Killburn, Kerry and Thomas Hutto. 1992. Process-Oriented Laboratory Activities for Introductory Biology. D. C. Heath and Company, Lexington, Massachusetts. 38 pp. (This is a laboratory manual with a hands-on, scientific method orientation for an introductory biology laboratory.)

Manney, Thomas R. and Monta L. Manney. 1991. Handbook for using yeast to teach genetics. Genetics Education Networking and Enhancement Project and National Science Foundation Teacher Enhancement Program. 86 pp. (This book contains many different yeast experiments. Pages 1-7 provide useful background information on yeast.)

Appendix A
Solutions for Yeast Fermentation Lab

Stock Solutions

1. Label several 2 liter (L) flasks with the proper stock solution name, using different colors of labeling tape for each solution type. Use the same color for all concentrations of a given solution.

2. Make the following quantities of stock solutions according to the directions given below.

Solution	Solute	Solvent	Total Quantity
20% Sucrose (table sugar)	200 g Sucrose	1 L Distilled Water	4 L
2.5% Na Lauryl Sulfate	25 g Na Lauryl Sulfate	1 L Distilled Water	1 L
2.5% Na Saccharin	25 g Na Saccharin	1 L Distilled Water	2 L
10% NaCl (table salt)	100 g NaCl	1 L Distilled Water	1 L
10% Starch (corn starch/amylose)	100 g Starch	1 L Distilled Water	2 L
60% Ethanol	600 mL Ethanol	400 mL Distilled Water	1 L

3. Label several 2 liter flasks with the correct solution name, using different colors of labeling tape for each solution type. Use the same color for all concentrations of a given solution.

4. Using stock solutions, make 1 liter of each of the following solutions according to the directions given below.

Sucrose Solutions

Solution	**20% Sucrose Stock**	**Distilled Water**
10% Sucrose	500 mL	500 mL
5% Sucrose	250 mL	750 mL
2.5% Sucrose	125 mL	875 mL

Starch Solutions

Solution	**10% Starch Stock**	**Distilled Water**
10% Starch	1 L	0 mL
5% Starch	500 mL	500 mL
2.5% Starch	250 mL	750 mL

Saccharin Solutions

Solution	2.5% Saccharin	Stock Distilled Water
2.5% Saccharin	1 L	0 mL
1.25 % Saccharin	500 mL	500 mL
0.67% Saccharin	250 mL	750 mL

NaCl/Sucrose Solutions

Solution	10% NaCl Stock	Distilled Water	20% Sucrose
10% NaCl/Sucrose	500 mL	0 mL	500 mL
5% NaCl/Sucrose	250 mL	250 mL	500 mL
2.5% NaCl/Sucrose	125 mL	375 mL	500 mL
1.25% NaCl/Sucrose	62.0 mL	438 mL	500 mL

Na Lauryl Sulfate(SLS)/Sucrose Solutions

Solution	2.5% SLS	Distilled Water	20 % Sucrose
2.5% SLS/Sucrose	500 mL	0 mL	500 mL
1.25% SLS/Sucrose	250 mL	250 mL	500 mL
0.67% SLS/Sucrose	125 mL	375 mL	500 mL
0.33% SLS/Sucrose	62.0 mL	438 mL	500 mL

Ethanol(EtOH)/Sucrose Solutions

Solution	60% Ethanol	Distilled Water	20% Sucrose
30% EtOH/Sucrose	500 mL	0 mL	500 mL
20% EtOH/Sucrose	333 mL	167 mL	500 mL
10% EtOH/Sucrose	167 mL	333 mL	500 mL
5% EtOH/Sucrose	82.5 mL	417.5 mL	500 mL

5. Label twenty-one 125 mL flasks using colored labeling tape. Color-code the flasks using the same color tape for each of the solution concentrations and types. For example: red - all sucrose; yellow - all NaCl/Sucrose. Each lab section (24 students) should have a complete set of solutions.

6. Dispense 100 mL of each solution into a separate, labeled 125 mL flask for each lab section. Note- if 10% sucrose is being used as a positive control, you should dispense 100 mL of this solution into 4 125 mL flasks for each lab section.

7. Cover solutions with Parafilm or use rubber stoppers.

8. Place prepared flasks on lab carts.

9. Check quantities after each lab section and refill if necessary.

10. Cover and store stock solutions in the refrigerator to prevent mold growth or evaporation.

11. Make yeast solution in 40Y C water immediately before each lab. Add 3 packages of rapid rise yeast to 1.4 liters of water. Add 30-50 g sucrose, stir and warm. Dispense 75 mL of yeast solution into flasks or bottles and set in 37° water baths about 15 minutes before lab starts.

Appendix B
Other Preparations

1. Fill two water baths approximately three-quarters full. Water should be almost to the tops of the test tubes when they are set in the racks. Set

temperatures to 45° and 37°.

2. Restock solutions following each laboratory section.

3. Turn off water baths at the end of the laboratory.

Appendix C
Doing this experiment without the BioLab computer program

1. The instructor can share all or selected parts of the data from the data base (Appendix D) after students have collected their own data from their own experiments.

2. The students then plot their data versus those provided in the data base from the instructor. This is exactly what the computer program does in the Validation section.

3. Students can also do the Prediction and Model sections of the experiments as follows:

A. Prediction

I. The student chooses one of the substrates and one of the temperatures for this "mental experiment" with the yeast.

II. The student writes the values of predicted data points for a specific substrate.

III. The student graphs his/her predicted data against those in the data base.

B. Model

I. Students use values from the data base to plot data for two substrates (on the same axes) under the specified conditions. The students then compare the plots and explain differences between the yeast's reactions with the two substrates.

4. If the instructor or students have access to a spreadsheet with a graphing function, they can duplicate many of the graphing features of the **BioLab** program.

Appendix D
Expected Outcomes

Sucrose

Yeast cells produce the enzyme sucrase, thus sucrose is a good substrate for fermentation. At lower concentrations, the reaction rate (as gauged by CO_2 production) is slower. At higher concentrations, the reaction rate may approach a plateau. Most students will pick sucrose as the best substrate for yeast. Instructors may wish to use sucrose as a control in experiments.

Sodium Chloride

At low concentrations, sodium chloride provides a stimulatory effect on fermentation. Bakers add salt to bread dough to stimulate the yeast's ability to raise the dough. At higher concentrations, sodium chloride has an inhibitory effect on fermentation, as indicated by the CO_2 output plateau after a lower volume. Some students will be able to discuss the osmotic effect of sodium chloride in higher concentrations on yeast.

Sodium Lauryl Sulfate

Sodium lauryl sulfate is a detergent. As such, it is an amphipathic molecule having polar and non-polar ends. This kind of molecule, found in shampoos and cleaners, dissolves cellular membranes. Yeast cells die in sodium lauryl sulfate. Many students have difficulty dealing with this concept in terms of their experimental results. (One group of students reported " if we had run our experiment longer, we think the yeast would have fermented the substrate.")

Starch

Many students assume that yeast will ferment starch "because it is a complex carbohydrate". Yeast do not produce amylase, however, and so do not ferment the starch and release CO_2.

Sodium Saccharin

Yeast, like humans, do not produce the enzymes to degrade saccharin, a synthetic sweetener. As a result, no CO_2 is produced. In many instances students will say that if they "waited longer" or "added more substrate" they would have seen fermentation.

Ethanol

A waste product of fermentation, ethanol acts to inhibit the reactions of fermentation, especially at higher concentrations. Some students will realize that ethanol shifts the equilibrium of the reaction.

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